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Bioactivation of organic nitrates and the mechanism of nitrate tolerance

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Abstract

Organic nitrates, such as nitroglycerin, are commonly used in the therapy of cardiovascular disease. Long-term therapy with these drugs, however, results in the rapid development of nitrate tolerance, limiting their hemodynamic and anti-ischemic efficacy. In addition, nitrate tolerance is associated with the expression of potentially deleterious modifications such as increased oxidative stress, endothelial dysfunction, and sympathetic activation. In this review we discuss current concepts regarding the mechanisms of organic nitrate bioactivation, nitrate tolerance, and nitrate-mediated oxidative stress and endothelial dysfunction. We also examine how hydralazine may prevent nitrate tolerance and related endothelial dysfunction. (Cardiol J 2009; 16: 11–19)

Key words: organic nitrates, nitrate tolerance, oxidative stress, endothelial dysfunction, hydralazine

Introduction

Organic nitrates, such as nitroglycerin (GTN, glycerol trinitrate), isosorbide dinitrate (ISDN), isosorbide mononitrate (ISMN), and pentaerithrityl tetranitrate (PETN), are commonly used in clinical cardiovascular medicine in the acute treatment of stable-effort angina, unstable angina, acute myocardial infarction, chronic congestive heart failure, pulmonary oedema, and severe arterial hypertension. The development of tolerance, i.e. the reduction in vasodilatatory effect or the requirement of higher doses that appear after continuous use, is a major factor limiting the efficacy of these drugs. Although intermittent nitrate therapy, which allows a daily nitrate washout interval, has been effective in the prevention of nitrate tolerance, this regimen is limited by its inability to provide a continuous and uninterrupted therapeutic effect [1–3].

Experimental and clinical investigations suggest that GTN-, ISDN-, and ISMN-induced tolerance is associated with the expression of potentially deleterious modifications such as increased oxidative stress, endothelial dysfunction, and sympathetic activation [4–6]. Therefore, there is increasing awareness of the fact that nitrate tolerance cannot simply be viewed as a loss of the beneficial effects of nitrate but also as a condition potentially mediating extra harmful consequences [7].

The exact mechanisms of nitrate bioactivation and the development of nitrate tolerance and nitrate-induced oxidative stress, endothelial dysfunction, and sympathetic activation are not completely understood [4, 8–10]. It is therefore not surprising that various strategies proposed for the prevention of nitrate tolerance, perhaps with the exception of hydralazine, have not proven particularly clinically beneficial. Hydralazine is a direct-acting smooth

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muscle relaxant with strong antioxidative properties, which, given simultaneously with nitrate, effectively prevents the development of oxidative stress and nitrate tolerance in experimental and clinical settings [8]. Chronic treatment with a combination of hydralazine and ISDN has been shown to have beneficial effects on left ventricular function and survival in patients with severe heart failure [11–13]. This clinical success of the chronic co-administration of nitrate and this antioxidant gives the prospect of a more effective means of chronic medication with organic nitrates. In this review we discuss current concepts on the mechanisms of nitrate bioactivation and tolerance. We also examine how hydralazine may prevent nitrate tolerance and endothelial dysfunction.

Basic pharmacology of organic nitrates

The beneficial clinical effects of GTN and other organic nitrates are due to preferential dilation of large conductance veins and large arteries while arterioles are dilated only by much higher concentrations of nitrates [14–16]. Low doses of organic nitrates cause peripheral venodilation, with redistribution of circulating blood volume away from the heart and lungs and toward the splanchnic and mesenteric circulation. The resulting reduction in preload is manifested by a decrease in cardiac chamber size, ventricular filling pressure and wall tension, and systemic blood pressure. Cardiac output in the normal heart may fall in response to nitrate administration, whereas cardiac output and stroke volume may show a modest rise in patients with left ventricular impairment.

Nitrates also induce arterial vasodilation that becomes progressively more marked with increasing dose and plasma concentration. Only at high plasma nitrate concentrations does arteriolar vasodilation occur. The increased arterial conductance and related decrease in peripheral vascular resistance lead to a reduction in left ventricular afterload.

The reduction in right and left ventricular preload and afterload caused by nitrate decreases cardiac work and lowers myocardial oxygen requirements. As a result, the ratio of myocardial oxygen demand to myocardial oxygen supply improves, and myocardial ischemia is alleviated or prevented.

A nitrate-induced increase in myocardial coronary flow (oxygen supply) is another important component of the anti-ischemic activity of these drugs. The effects of nitrates on coronary circulation include:

— improvement in the subendocardial/subepicardial blood flow ratio; the mechanism of effort-angina involves an exercise-induced increase in subepicardial coronary flow and a simultaneous decrease in the subendocardial flow in the heart muscle territory supplied by the stenotic coronary artery [17]. This redistribution of flow from endocardium to epicardium is attenuated or prevented by organic nitrates;
— prevention or reversal of coronary artery vasoconstriction — spontaneous or precipitated by exercise; eccentric atherosclerotic lesions with a rim or arc of intact vascular smooth muscle remaining in the arterial wall are capable of contraction and relaxation, and paradoxical vasospasm of this region is often precipitated by exercise. Coronary artery relaxation induced by organic nitrates may increase the calibre of these stenoses, thereby decreasing resistance across the obstruction and improving the coronary flow;
— dilatation of coronary collateral vessels.

In addition to vascular action, organic nitrates have antiaggregatory properties in patients with stable and unstable angina.

In summary, it is believed that the combination of increased supply and decreased demand of oxygen is a unique therapeutic benefit of organic nitrates in cardiac ischemia. In addition, because of their hemodynamic profile, nitrates may be particularly useful in patients with angina who have impaired left ventricular systolic function or heart failure.

GTN and other nitrates function as prodrugs that, when bioactivated, release nitric oxide (NO) or S-nitrosothiol in vascular smooth muscle and endothelial cells. This nitrate-derived NO eventually supplements endothelial production of NO. In this sense, nitrates are endothelium-independent vasodilators that are not reliant on functioning endothelium for their vasodilator activity. It is believed, therefore, that the same downstream cellular mechanism accounts for the vasorelaxant bioactivity of organic nitrates, vasorelaxation by endothelium-dependent vasodilators (agonists like acetylcholine that stimulate endothelial NO formation) and by direct NO donors (e.g. nitroprusside), and for the basal endothelium-dependent vasodilatory tone present in the vasculature (Fig. 1, right-hand side).

Thus, NO activates an intracellular NO receptor enzyme, soluble guanylyl cyclase (sGC), increasing tissue levels of the second messenger cyclic 3'5'-guanosine monophosphate (cGMP). This results in the activation of cGMP-dependent protein kinase (PKG) and cGMP-gated ion channels. The phosphorylation of target proteins by PKG eventually mediates smooth muscle relaxation and other

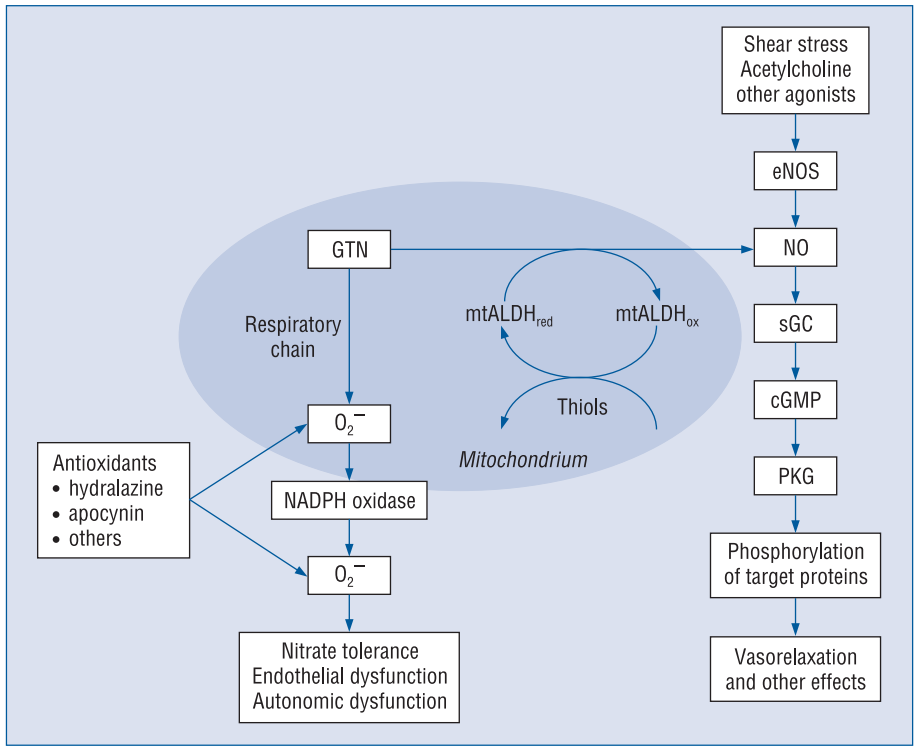


Figure 1. Biological effects accompanying biotransformation of nitroglycerine (GTN). **Right-hand side:** GTN is biotransformed by mitochondrial aldehyde dehydrogenase (mtALDH) to release nitric oxide (NO), and NO activates sGC-cGMP-PKG signal transduction mechanism to produce vasodilation. The same vasodilation transduction mechanism is used by factors that activate endothelial NO synthase (eNOS) and increase endothelial NO formation by this enzyme. Shear stress-mediated activation of eNOS and related basal NO production maintain a basal endothelium-dependent vasodilator tone in the vasculature. Endothelial receptor activation and related eNOS stimulation and NO production account for vasodilation produced by a number of agonists. **Left-hand side:** Simultaneously, GTN uncouples the mitochondrial respiratory chain to increase mitochondrial superoxide anion production (O_2^-). This results in the activation of membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. O_2^- produced by this enzyme causes a number of adverse effects, which include endothelial and autonomic dysfunction, and oxidation of a critical thiol group in the active site of mtALDH, resulting in impaired GTN biotransformation and nitrate tolerance. For further explanation see text; sGC — soluble guanylyl cyclase; cGMP — cyclic guanosine-3′, 5′-monophosphate; PKG — cGMP activated protein kinase.

cellular effects. The mechanism of vasodilation includes cGMP- and PKG-mediated attenuation of an agonist-mediated rise in intracellular Ca^{2+} levels and desensitization of contractile proteins to Ca^{2+} in smooth muscle cells [4, 18]. This hypothesis on sGC-cGMP-PKG vascular signalling pathways of nitrates is supported by the findings that the inhibition of NO-sensitive sGC attenuates nitrate-mediated vasorelaxation and that the inhibition of phosphodiesterases that degrade cGMP increases the vasorelaxation potency of nitrates [4, 9, 19].

Bioactivation of organic nitrates

Pathways that are of a degrading and activating nature metabolize organic nitrates. It is believed that the bioactivation routes involve the production of glycerol-1,2-dinitrate, inorganic nitrite, and finally S-nitrosothiols and NO (Fig. 2). There has been an extensive search for the nonenzymatic and enzymatic reactions catalyzing GTN bioactivation and several candidates have been proposed, including thiol-supported GTN bioactivation, GSH-S-transferase, cytochrome P450, cytochrome P450 reductase, and xanthine oxidase [4, 10]. However, none of these enzymatic pathways appears to satisfactorily explain the therapeutic activity of organic nitrates, as they all require concentrations of nitrates approximately 3–4 orders of magnitude higher than those reached in plasma during most clinical applications (100–1000 μM range *vs.* < 50 nM measured in patients [4, 19]).

Recently Chen at al. [20] provided evidence that the mitochondrial isoform of aldehyde dehydrogenase (ALDH-2 or mtALDH) is responsible for the bioactivation of GTN. The authors demonstrated

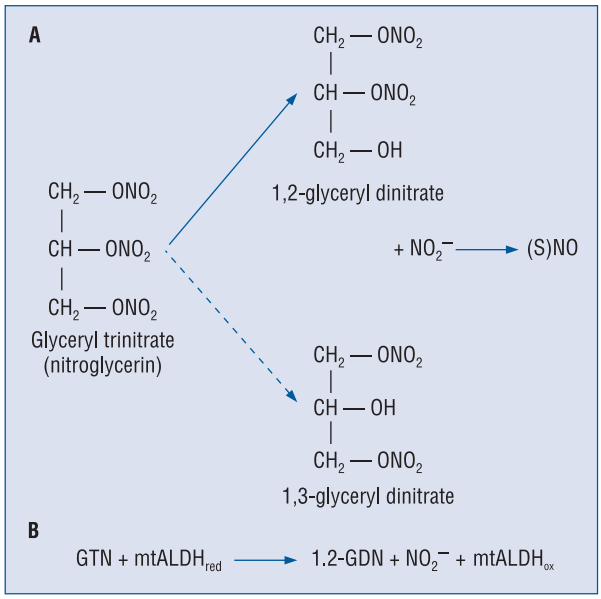


Figure 2. Cellular metabolism of low levels of nitroglycerin (GTN) in blood vessels. **A.** Bioactivation routes of GTN are catalyzed by mtALDH and yield 1,2-dinitrate and nitrite, and result in generation of NO and/or S-nitrosothiol, and in vasodilatory activity. Degradation routes yield predominantly 1,3-dinitrate + nitrite, rather than 1,2-dinitrate + nitrite; **B.** The overall reaction of mtATDH catalyzed bioactivation of GTN. The reaction involves the reduction of the nitrogen in the dissociated nitrite (reductase activity of mfALDH). The electrons for this reduction are provided by cysteine-thiols at the active site of the enzyme that are thereby converted to a disulfide. Thereby, the enzyme undergoes reversible conversion from its reduced active form to its oxidized inactive form. Reductase activity of mtALDH can be restored by dithiol compounds, explaining why bioactivation of nitrates is strongly dependent on thiol bioavailability and why exogenous thiols prevent nitrate tolerance [9, 10, 19]. However, an oxidative attack of reactive oxygen species and reactive nitrogen species on thiol components of mtALDH may result in irreversible inhibition of the enzyme [10].

that purified mtALDH specifically catalyses the formation of 1,2-glycerol dinitrate and nitrite from GTN (used in small clinically relevant concentrations) (Fig. 2), that this reaction is supported by various dithiols, that it mediates the production of cGMP and the relaxation of vascular smooth muscle, and that all these effects of GTN are eliminated by mtALDH inhibition or genetic deletion of mtALDH [21]. These studies demonstrated that mtALDH is necessary and sufficient for vasoactivity derived from therapeutic levels of GTN. In this context the authors have speculated that nitrite generated within the mitochondria is metabolized further to generate NO-based bioactivity by reduction to NO and/or by conversion to S-nitrosothiol [19, 20] (Fig. 2). Indeed, it has recently been demonstrated that mtALDH catalyzed GTN conversion leads to NO generation [9]. However, the exact reaction mechanism of this mitochondrial NO production, as well as the form in which NO bioactivity is conveyed from mitochondria to cytosolic sGC, remain unresolved [9, 19].

It has been demonstrated further that the inhibition of mtALDH results in the bioactivation inhibition of GTN and PETN but not ISDN and ISMN, consistent with the bioactivation of the isosorbide nitrovasodilators by a mtALDH independent pathway [22].

It is well known that GTN and ISDN can inhibit mtALDH activity [23]. However, Chen et al. [20] were the first to provide evidence that prolonged treatment with GTN results in GTN tolerance and simultaneous inhibition of mtALDH in vascular preparations (Fig. 2), and that the inhibition of mtALDH with various specific enzyme inhibitors results in vascular GTN tolerance, indicating that attenuated nitrate bioactivation by mtALDH underlines, at least partially, the induction of nitrate tolerance.

mtALDH and nitrate-alcohol interactions

mtALDH is a major enzyme in the detoxification of acetylaldehyde produced during ethanol oxidation (it catalyzes acetylaldehyde oxidation to form acetic acid). About 40% of the East-Asian population that expresses a low-activity mutant of mtALDH (Glu487Lys) exhibits significantly lowered alcohol tolerance (due to adverse effects of acetylaldehyde accumulation) as well as decreased sensitivity to nitroglycerin treatment [19, 24–26]. On the other hand, organic nitrate medication along with alcohol consumption causes disulfiran-like reactions, including blurred vision, nausea, and flushing of the face and neck. Disulfiran (Anticol) is an ALDH inhibitor that is still approved in many countries as a deterrent for the aversive pharmacotherapy of alcohol dependence. Recently disulfiran has been demonstrated to induce GTN-tolerance and endothelial dysfunction in healthy volunteers [27]. Altogether these observations imply that mtALDH plays a role in GTN bioactivation also in humans.

Tolerance, cross-tolerance, and tachyphylaxis to organic nitrates

The phenomenon of tolerance is characterized by impaired responsiveness to the organic nitrate,

which is, in turn, demonstrated by reduced nitrate-induced vasodilatation, reduced nitrate-induced blood pressure lowering effect, and/or attenuation of nitrate-induced anti-ischemic effect. Nitrate-induced tolerance to other endothelium dependent (e.g. acetylcholine) and endothelium-independent nitrovasodilators (e.g. nitroprusside) is termed cross-tolerance. Tachyphylaxis denotes impaired nitrate potency in response to short-term challenges of vessels, usually with a high concentration of nitrate [1–3].

The tolerance has been reported to occur within 1–3 days of continuous GTN treatment in patients with acute myocardial infarction [28] stable-effort angina, and chronic congestive heart failure [29].

Clinical studies have shown that signs of tolerance also develop with chronic administration of ISDN and ISMN, and that both these drugs also induced severe endothelial dysfunction and oxidative stress with chronic treatment [30, 31]. PETN seems to be an exception among nitrates as it was reported to produce continuous vasodilation without signs of tolerance in rabbits [32] and in humans [33, 34]. In addition, in contrast to GTN, PETN caused neither oxidative stress in humans [34] nor endothelial dysfunction in hypercholesterolemic rabbits [35]. It was suggested that these might be due to the fact that PETN induces the antioxidant defence protein heme oxygenase producing antioxidant molecule bilibubin and the vasodilator carbon monoxide [22].

Mechanism of nitrate tolerance

The mechanisms underlying the phenomenon of nitrate tolerance are still poorly defined. Recent animal and human studies indicate that increased vascular production of superoxide anion (O_2^-) underlies the mechanism of the tolerance. In addition, short-term experimental and clinical investigations suggest that oxidative stress associated with tolerance induced by nitrate causes potentially deleterious modifications such as endothelial dysfunction and sympathetic activation, which partially add to the mechanism of nitrate tolerance and can be viewed as additional side-effects of organic nitrate therapy [5–7].

Free radical hypothesis of nitrate tolerance

In 1995 Münzel at al. [36] were the first to report that the sustained administration of GTN causes increased vascular formation of O_2^-. Later the same group identified the mitochondria as a major source of oxidative stress in tolerant animals [22, 37], a finding confirmed by others [38]. These findings led to the oxidative stress hypothesis of nitrate tolerance [4, 6–8, 10].

Recent evidence suggests that the mitochondrial respiratory chain is the primary source of the nitrate-induced O_2^- overproduction in vessels, leading to a subsequent activation of vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and that mostly NADPH oxidase-derived O_2^- mediates nitrate tolerance and endothelial dysfunction (Fig. 1, left-hand side) [39].

The superoxide anion is normally scavenged by various intracellular and extracellular mechanisms, including the enzyme superoxide dismutase. However, in higher concentrations it can overcome these mechanisms and rapidly react with NO (that produced by the endothelium and nitrate-derived) to form strong oxidant peroxynitrite [40]. These reactive oxygen species (ROS) are believed to cause nitrate tolerance in at least 3 mechanisms [4, 9, 10]:

- formation of peroxynitrite may reduce the bioavailability of nitrate-derived NO, impairing its vasodilator activity (and of other nitrodilators);
- vascular oxidative stress may cause sGC and PKG inhibition, leading to desensitization of vasodilator signalling pathway of organic nitrates and of other nitrovasodilators;
- mitochondrial ROS were shown to inactivate mtALDH, suggesting that reduced bioactivation of nitrates may underlie, at least partially, the mechanism of nitrate tolerance.

The oxidative stress hypothesis of nitrate tolerance is supported by numerous reports demonstrating that the tolerance is prevented, or at least ameliorated, by co-administration of various antioxidants (e.g., vitamin C, vitamin E, folic acid, beta-adrenergic antagonist carvedilol, the vasodilator hydralazine) and interventions inhibiting NADPH oxidase-dependent ROS formation (statins, ACE inhibitors, hydralazine and NADPH oxidase inhibitor apocynin) [4, 8–10, 39]. Interestingly, all of these antioxidative interventions are also known to protect against the endothelial dysfunction caused by various oxidative stress-related cardiovascular disorders [41, 42], providing strong evidence for O_2^--mediated inactivation of NO as a common mechanism of nitrate tolerance and endothelial dysfunction.

Nitrate-induced endothelial dysfunction

Impaired endothelium-dependent vasodilation, as evidenced by impaired flow-mediated and/or acetylcholine-mediated vasodilation and simultaneously preserved vasodilation to direct NO donor (e.g. GTN or nitroprusside), is a hallmark of endo-

thelial dysfunction. Continuous GTN treatment is associated with oxygen-free radical induced worsening of endothelial function in patients with coronary artery disease [31, 43, 44] and even induction of endothelial dysfunction in healthy subjects [45].

Endothelial dysfunction my be an important deleterious complication of nitrate medication as it is a major predictor of cardiac events in patients with coronary artery disease [46–48] and heart failure [49]. In this context, a meta-analysis of clinical studies suggested that nitrate tolerance may underline the increases in cardiac morbidity seen with chronic nitrate use [50]. However, generally there is scant evidence concerning the long-term clinical outcome of nitrate therapy. This is mainly because the largest studies of organic nitrates in the setting of stable angina pectoris or in post-infarction patients (GISSI, ISIS) involved follow-up periods of only few weeks [7]. This emphasizes the need for large-scale, controlled studies regarding the efficacy and safety of chronic nitrate therapy in coronary artery disease.

Neuro-hormonal counter-regulation and autonomic dysfunction

Medication with any vasodilator (e.g., organic nitrate, dihydropiridine calcium channel blocker, hydralazine) may be associated with counter regulatory neurohormonal activation initiated by drop in blood pressure, and related arterial baroreceptor unloading. In the case of organic nitrates, this has been demonstrated in patients with coronary heart disease [51] heart failure [52] and healthy volunteers [53] given GTN in doses producing a drop in blood pressure. The reaction included increases in blood levels of catecholamines [53], renin and aldosterone [51, 53], and vasopressin [51, 53]. Probably as a result of this neuro-hormonal stimulation, GTN therapy is associated with water and sodium retention, weight gain and a marked increase in intravascular volume (as evidenced by a decrease in hematocrit), which counteract the blood pressure and preload lowering effects of GTN [51, 53]. By contrast, intermittent transdermal GTN therapy was associated with a different pattern of hormonal response (values tending to return to baseline levels after the nitrate-free interval), a lack of sodium retention, and no evidence of plasma volume expansion [53].

The loss of the initial hypotensive response after continuous, but not intermittent, GTN administration has been repeatedly reported as evidence of the occurrence of GTN tolerance [53]. Currently this phenomenon is termed nitrate pseudo-tolerance because at the time when the nitrate hypotensive response ceases the vascular responsiveness to nitrate-induced vasodilation is still preserved [4, 51].

Besides the early neuro-hormonal counter-regulatory mechanism mediated by the initial blood pressure drop, chronic GTN treatment also results in persistent autonomic dysfunction that is evident even after the normalization of blood pressure. Thus, prolonged exposure to GTN has been shown to shift the physiological balance between the sympathetic and vagal nervous systems in the modulation of cardiac heart rate and heart rate variability towards a prevalence of the sympathetic system, impairing baroreflex function, and heart rate variability [5]. Such modifications might have negative prognostic implications as they have been associated with increased incidence of arrhythmias and worse prognosis in patients with coronary artery disease and heart failure [54].

It has been proposed that nitrate-mediated autonomic dysfunction is caused by reduction of endogenous NO availability and/or synthesis in the central nervous system as a result of oxidative stress caused by continuous treatment with nitrate, and that this process would be analogous to abnormalities in vascular endothelial NO metabolism. NO synthesis in the brain stem appears to have a chronic inhibitory effect on medullary areas modulating sympathetic outflow. This effect may be lost with prolonged nitrate treatment and subsequent development of oxidative stress [5–7].

Co-administration of hydralazine and nitrate

Hydralazine hydrochloride is a direct-acting smooth muscle relaxant used to treat hypertension by acting as a vasodilator primarily in arterioles. The cellular mechanism of vasodilation by hydralazine is not well known. Hydralazine is not used as a primary drug for treating hypertension because it elicits a reflex sympathetic activation that, by increasing heart rate and cardiac output, may cause myocardial ischemia. Hydralazine may also increase plasma renin concentration, resulting in fluid retention. In order to prevent these undesirable side effects, hydralazine is generally prescribed in combination with a beta-blocker and a diuretic [55].

While organic nitrates mainly dilate large capacitance veins and large conductance arteries, hydralazine is a potent arteriolar dilator. Therefore, the initial rationale for use of organic nitrates and hydralazine in combination in heart failure was their complementary hemodynamic effect caused by

the predominant venodilatory and preload reducing effect of nitrates and the arteriolar-dilatory and afterload reducing effect of hydralazine. This hemodynamic rationale was the basis for the Vasodilator Heart Failure Trial (V-HeFT), which evaluated the effect of the combination of ISDN and hydralazine on the outcome of patients with chronic heart failure [56, 57]. In the first part of that study the combination ISDN/hydralazine resulted in a significant reduction in mortality compared with results in patients treated with either placebo or prazosin [56]. The V-HeFT II study, designed to compare the effects of the ISDN/hydralazine combination with ACE-inhibitor enalapril, revealed that enalapril had a greater effect on survival than the combination ISDN/hydralazine. However, the combination ISDN/hydralazine improved exercise tolerance and left ventricular ejection fraction more than enalapril in these patients [57]. More recently, a fixed dose combination of ISDN/hydralazine added to a standard background neurohormonal blockade has been shown to decrease mortality, reduce the incidence of first hospitalization for heart failure, and improve quality of life in the African American Heart Failure Trial (A-HeFT) [58, 59].

Hydralazine, similarly to organic nitrates, mediates counter regulatory neurohormonal activation, as evidenced by increases in plasma catecholamines and renin activity. Theoretically, this would be expected to aggravate counter regulatory adjustments to nitrate and worsen nitrate tolerance resulting from frequent administration of ISDN [30, 31].

Nonetheless, hydralazine was shown to prevent GNT tolerance in experimental models [60–62]. Furthermore, hydralazine co-administered with GTN appeared to prevent early development of GTN tolerance and resulted in a persistent GTN-mediated beneficial hemodynamic effect on systemic and pulmonary artery and left ventricular filling pressure in patients with chronic systolic heart failure [63]. In addition, it has been reported that hydralazine: inhibits vascular NADPH oxidase, prevents nitrate-induced vascular O_2^- overproduction [62], and normalizes impaired PKG and impaired mtALDH activity in nitrate-tolerant vessels [64]. Therefore, the current belief is that hydralazine prevents nitrate tolerance because it has strong antioxidative properties, and that prevention of nitrate tolerance with hydralazine enables the maintenance of the favourable hemodynamic effects of nitrates [8, 65]. However, hydralazine may offer an additional therapeutic benefit inherent to hydralazine antioxidant effects [64].

Summary

There is mounting evidence that systemic therapy with GTN, ISDN, and ISMN, but not with PETN, induces tolerance and endothelial dysfunction, and that these effects are a major limitation of the therapy with these drugs. Mitochondrial ALDH has recently been recognized as an enzyme that bioactivates GTN and PETN to produce NO. ISDN and ISMN seem to undergo bioactivation by an mtALDH-independent pathway. Bioactivation of GTN (and probably of ISDN and ISMN, but not of PETN) also results in the uncoupling of the mitochondrial respiratory chain that leads to increased mitochondrial O_2^- production, which further mediates the activation of NADPH oxidase and increased O_2^- production by this enzyme. The most likely mechanism of nitrate tolerance involves O_2^--induced inhibition of mtALDH, scavenging of nitrate-derived NO, and the inhibition of various steps in NO vasodilator signalling pathway. Oxidative stress also causes endothelial dysfunction and probably sympathetic activation; two consequences that may be an additional risk for patients who receive continuous nitrate medication to treat conditions such as stable and unstable angina or heart failure. However, the prognostic effect of nitrates in stable coronary artery disease has never been studied systematically, and "early entry" therapy with nitrates does not significantly improve survival in myocardial infarction although it increases the beneficial effects of the ACE-inhibitor enalapril by 50% [16]. By contrast, these toxic phenomena might be offset by the use of drugs that have antioxidant properties and/or prevent nitrate-induced oxygen free radical production. This notion is supported by clinical studies demonstrating the beneficial effects of ISDN combined with hydralazine in patients with chronic heart disease.

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